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Editorial: A New Face for an Old Syndrome

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INTRODUCTION

The description in 1993 of abnormal cholesterol metabolism in patients with RSH/Smith-Lemli-Opitz syndrome (RSH/SLOS) [Irons et al., 1993] marked a discovery with major implications not only for the diagnosis and treatment of patients with RSH/SLOS, but also for our conceptualization of inborn errors of metabolism as causes of malformation syndromes. As is often the case in science, a key finding, namely, increased blood and tissue levels of 7-dehydrocholesterol (7DHC) in patients with RSH/SLOS, brought into focus knowledge from fields as diverse as clinical genetics, sterol biochemistry, experimental teratology, and fetal nutrition, and fostered a rush of new insights. Although there are other syndromes, such as Zellweger syndrome, comprising malformations and pathological biochemical abnormalities, RSH/SLOS is perhaps the best example yet of a syndrome in which a discrete block in a single metabolic pathway leads to severe malformations. As such, RSH/SLOS presents us with an unusual opportunity to examine the relationship between biochemistry and abnormal morphogenesis.

The series of articles in this issue of AJMG continues this rapid expansion of knowledge about RSH/SLOS. Most of the papers and abstracts that follow were presented or developed from talks given at an NICHD conference devoted to RSH/SLOS held September 26–27, 1995, in Bethesda, Maryland. The conference was organized by the discoverer of RSH/SLOS, Dr. John Opitz, and generously sponsored by Dr. Felix de la Cruz of NICHD. During a packed 2 days of lectures and discussion, more than 30 scientists and clinicians met to discuss their recent work. In the next few pages, I will attempt to summarize the essence of the meeting and the many new discoveries and observations made about RSH/SLOS as a fundamental defect of sterol metabolism, as a newly recognized biochemical malformation syndrome, and as a clinical management problem requiring urgent solution.

STEROL BIOCHEMISTRY IN RSH/SLOS

As shown first by Tint et al. [1994], the fundamental biochemical abnormality in RSH/SLOS is an increased level of 7DHC in blood and tissues. Several studies, including those by Ness et al. [1997], Tint et al. [1997], Cunniff et al. [1997], and Honda et al. [1997] in the following pages, demonstrate clearly how all tissues and body fluids reflect this sterol abnormality. Although the sterol pattern in SLOS plasma is complex and contains isomers of 7DHC as well as other sterols that are precursors or derivatives of 7DHC, the pattern is consistent with a block in the last step of cholesterol biosynthesis, i.e., the reduction of 7DHC to cholesterol (Fig. 1). This conclusion has also been supported by the observation that normal fibroblasts grown in the presence AY-9944 and BM 15.766, relatively specific inhibitors of 7DHC reductase, have sterol profiles essentially identical to those of RSH/SLOS fibroblasts, as also described in this issue by Honda et al. [1997] and in an earlier report from the same laboratory [Xu et al. 1995].

Preliminary enzymatic studies by Shefer et al. [1995] demonstrated a marked deficiency of 3β-hydroxysteroid-Δ^7-reductase (7DHC reductase), the microsomal enzyme that converts 7DHC to cholesterol. Although a deficiency of the enzyme itself is suspected, very little is known about the structure or regulation of 7DHC reductase and the role of associated proteins, such as sterol carrier protein 2 (SCP2). At this time, a *genetic* deficiency of 7DHC reductase has not been proven, but work on the purification of the enzyme and its characterization in skin fibroblasts and other tissues is in progress. Several laboratories have looked for a deficiency of SCP2 by immunological means in RSH/SLOS fibroblasts, but all have found normal antigen levels.

Although the original observations of Irons et al. [1993], Tint et al. [1994], and Natowicz and Evans [1994] were essential elements in the discovery of defective cholesterol biosynthesis in RSH/SLOS, it is interesting to note the many lines of evidence that, prior to 1993, pointed to an abnormality of sterol metabolism in RSH/SLOS. For example, Chasalow et al. [1985] described a consistent, abnormal steroid pattern in RSH/SLOS comprising several unusual steroids, each monounsaturated relative to the normally abundant steroids. However, these investigators apparently did not look at cholesterol or its precursors. Several years later, in the original study delineating RSH/SLOS "type II" [Curry et al., 1987], severely depressed levels of HDL and especially LDL cholesterol were reported in one patient. More recently, McKeever and Young [1990] reported finding very low maternal blood levels of estriol, a fetal sterol product, in an RSH/SLOS pregnancy and speculated that RSH/SLOS may be caused by a pri-

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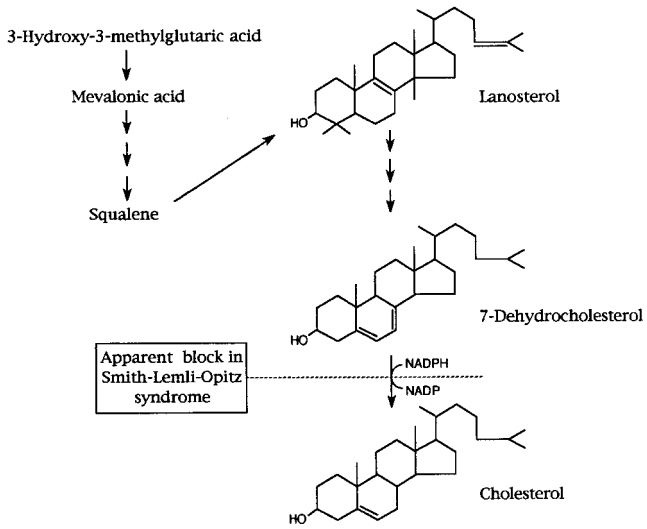

Fig. 1. Biosynthesis of cholesterol.

mary abnormality of sterol metabolism. Long before any of these studies, Roux and other French scientists [Roux and Aubry, 1966; Roux et al., 1980; Barbu et al., 1988] studied the teratogenic effect of several inhibitors of sterol synthesis, including agents that block 7DHC reductase, and found a number of malformations in common with those of RSH/SLOS. Thus, clues to the pathogenesis of RSH/SLOS had been published over a span of almost 30 years before the discovery of increased levels of 7DHC in RSH/SLOS. Knowing that a simple clue, i.e., hypocholesterolemia, to such an important discovery has probably passed before our eyes many times should prompt us as clinical geneticists to think more carefully about peculiar laboratory abnormalities in patients with biochemically unexplained genetic syndromes.

CLINICAL SPECTRUM OF RSH/SLOS SYNDROME AND ABNORMAL CHOLESTEROL METABOLISM

One of the first clinical questions to be asked following the discovery of defective sterol metabolism in RSH/SLOS was whether or not all patients with a clinical diagnosis of RSH/SLOS have the same biochemical abnormality, and, as a corollary, whether or not there is a biochemical difference between mild (type I) and severe (type II) RSH/SLOS. The first question has been more difficult to answer than the second because of the previously subjective nature of the diagnosis of RSH/SLOS. However, as noted by Cunniff et al. [1997], a comparison of the frequency of anomalies in published cases vs. biochemically abnormal cases of RSH/SLOS suggests that up to 25% of published cases may be genetically different disorders. On the other hand, it is rare in our laboratory, having occurred only 2 or 3 times out of the first 100 biochemically identified RSH/SLOS patients, that a patient with a firm clinical diagnosis of RSH/SLOS made by a seasoned geneticist has had apparently normal sterol metabolism in both blood and cultured cells. The same paper also answers clearly the second question about differences in clinical severity. In that study of 80 biochemically positive cases, all patients, ranging from those with only mental retardation and second and third toe syndactyly to severely deformed fetuses with lethal internal anomalies, had qualitatively the same abnormality of sterol metabolism. Surprisingly, however, clinical severity correlated not with the increased level of 7DHC but inversely with the cholesterol level at diagnosis. Biochemical findings in severely affected RSH/SLOS patients are also described in this issue by Faes et al. [1997], Cormier-Daire et al. [1997], and Ness et al. [1997]. Another unusual presentation of RSH/SLOS with increased 7DHC levels has been holoprosencephaly [Muenke et al., 1994], one of the more common malformations found in the offspring of rats treated with inhibitors of 7DHC reductase [Roux and Aubry, 1966]. Although only one RSH/SLOS patient with alobar holoprosencephaly has been reported, less complete forms of the holoprosencephaly sequence, such as midline cleft lip, have been documented several times in RSH/SLOS [McKeever and Young, 1990; Kelley, unpublished observations].

RSH/SLOS POPULATION GENETICS AND EPIDEMIOLOGY

Early epidemiologic studies in British Columbia estimated the incidence of RSH/SLOS to be about 1 in 20,000 births in a population of largely Anglo-Saxon and Northern European descent [Lowry and Yong, 1980]. However, Opitz et al. (unpublished observations), in this issue, estimate an incidence of RSH/SLOS of greater than 1 in 10,000 births in a completely ascertained newborn population in Middle Bohemia (Czech Republic) *before* biochemical testing was available. One of the reasons for the unusually high incidence in the Bohemian cohort may be that more than half of the cases considered definite were perinatal or neonatal deaths, a proportion that far exceeds the 20–25% fetal/perinatal death rate observed in our laboratory (Kelley, unpublished observations).

With the availability of a simple yet apparently definitive biochemical test for RSH/SLOS, and with the evident expansion of the RSH/SLOS phenotype at both extremes of severity, the estimated incidences in different populations may need to be revised upwards, even considering the estimate [Cunniff et al., 1997] that 25% of cases may be genetically different diseases. On the other hand, between the two laboratories where probably more than 90% of the diagnoses in the United States have been made, the rate of new diagnoses in children under age 1 year has been only about 40 per year, or less than one in 50,000 births (Kelley and Tint, unpublished data). Thus, either many new cases are still being missed in some communities, or there may be substantial population differences in the incidence of RSH/SLOS, with a much higher frequency among patients of Northern European ancestry. Indeed, despite the great admixture of ethnic groups in the United States, only one out of 150 biochemically positive cases has had African ancestry and none Asian (Kelley and Tint, unpublished observations). A relatively high carrier frequency of RSH/SLOS, at least among Northern

Europeans, is suggested by the infrequent consanguinity of RSH/SLOS parents, as reported in this issue [Cunniff et al., 1997] and as noted in earlier studies of RSH/SLOS [Opitz et al., 1969; Johnson, 1975; Jeanty et al., 1977]. Moreover, in 4 of the first 65 RSH/SLOS index families identified biochemically by our laboratory, there were other affected relatives, i.e., cousins in two families and uncles/aunts in two other families. In three of these secondary sibships, the defect was confirmed biochemically, and in the fourth the diagnosis of RSH/SLOS was clinically unmistakable.

Although the attractive hypothesis of heterozygote advantage via lowered blood cholesterol levels is often mentioned [Opitz and de la Cruz, 1994], complicated modifier gene or major environmental or dietary effects would have to be postulated to explain the highly variable population incidence of RSH/SLOS in different ethnic groups. Recent population studies of medium-chain acyl-CoA dehydrogenase (MCAD) in Europe [Andresen et al., 1995] documented an unusually high carrier frequency, as high as 1 in 60, of the common G985 MCAD mutation (80% of mutant alleles), a relatively recent mutation in Northwestern Europe associated with a specific haplotype. Perhaps a prevalent RSH/SLOS mutation arising in Northern Europe by a similar founder effect will be found to explain a large proportion of RSH/SLOS cases. Although multiple genetic causes of the RSH/SLOS biochemical phenotype may exist, the remarkable frequency of secondary sibships and the rare occurrence of consanguinity together argue for a high carrier frequency of a single RSH/SLOS genetic locus causing the large majority of RSH/SLOS cases.

Clearly, much more needs to be known about the genetics of RSH/SLOS. Perhaps only when the RSH/SLOS gene (or genes) is identified will we be able to explain the variations in population incidence. In the accompanying paper by Alley et al. [1997], progress on the definition of a possible RSH/SLOS gene on chromosome 7 is described. With an excellent candidate locus (based on different balanced translocations at 7q32 in 2 RSH/SLOS patients [Alley et al., 1995; Curry et al., 1987; Wallace et al., 1994]), and the isolation of a small DNA segment containing the breakpoint region [Alley et al., 1995], identification of the RSH/SLOS gene is probably not far off. In the meantime, methods of newborn screening for RSH/SLOS, such as time-of-flight secondary ion mass spectrometry (TOF-SIMS) technique described in this issue [Zimmerman et al., 1997], may go a long way in answering important genetic and epidemiologic questions. As first developed by Zimmerman et al. [1997] and now used by others [Seedorf et al., 1995], TOF-SIMS allows screening for RSH/SLOS by determining the 7DHC/cholesterol ratio in the same Guthrie blood card specimen used for newborn metabolic screening. The technique appears to be sensitive and adaptable to large-volume screening. For the existing population, efforts should also be directed toward identifying atypical RSH/SLOS patients at both extremes of severity. Thus, screening for RSH/SLOS among undiagnosed patients in clinics for cataracts, cleft palate, and hypospadias should be undertaken. Because of the apparent critical role of hypocholesterolemia in the pathogenesis of RSH/SLOS malformations, such biochemical screening should be extended to include cholesterol precursors other than 7DHC.

PRENATAL AND POSTNATAL METHODS FOR DIAGNOSIS OF RSH/SLOS

Because of the great clinical variability of RSH/SLOS and the ability to identify many new RSH/SLOS patients who do not satisfy pre-1993 clinical criteria for the diagnosis, there must be wider knowledge and use of sterol analysis to identify all affected RSH/SLOS children and adults. Fortunately, cholesterol, 7DHC, and their metabolites are present in all body tissues and fluids, almost any of which can be used for diagnosis. Although, as described in many of the articles in this issue, measurement of the 7DHC level in blood is the most common route to a biochemical diagnosis of RSH/SLOS, many other sample types have been used for both current and retrospective diagnoses. Amniotic fluid, chorionic villus, fetal tissues (both frozen and formalin-preserved), fibroblasts, lymphoblasts, amniocytes, cultured villus, and archived newborn screening blood cards have all been used more than once to make a diagnosis of RSH/SLOS. Considering the many laboratory tests that are often performed on patients suspected of having a genetic syndrome, an informative stored sample can often be found when a retrospective diagnosis is needed. Perhaps the most striking retrospective diagnosis of RSH/SLOS was that of a 130-year-old museum specimen of an infant "Hermaphroditismus Polydactylie" from Vrolik's collection, as described in this issue by Oostra et al. [1997]. Even though the specimen was far too old to harbor diagnostic levels of 7DHC, a relatively unstable sterol, the level of cholesterol in the preserved tissue was much below that of an unaffected infant from the same era in the Vrolik collection.

Prenatal diagnosis of RSH/SLOS by biochemical testing has been one of the most important dividends of our new understanding of RSH/SLOS. Amniotic fluid from an affected RSH/SLOS pregnancy offers one of the highest differentials in prenatal diagnosis, with levels of 7DHC typically 1,000–5,000-fold increased over controls [Abuelo et al., 1995; Kelley, 1995; Rossiter et al., 1995]. Despite several publications on methods for prenatal diagnosis of RSH/SLOS and on the significance of low maternal serum levels of estriol [McKeever and Young, 1990; Rossiter et al., 1995], screening for RSH/SLOS in pregnancies with low maternal estriol levels or sonographically identified RSH/SLOS malformations is rarely requested. As a result, many RSH/SLOS pregnancies may be proceeding to term or terminating prematurely without a diagnosis.

Several papers in this issue also describe studies of cultured skin fibroblasts and the use of cultured RSH/SLOS cells for explorations of the biochemistry of SLOS/RSH. As shown by Honda et al. [1997], an older patient with minimally increased levels of 7DHC had essentially the same abnormal 7DHC/total sterol ratio as more classically affected individuals when his skin fibroblasts were grown in delipidated culture medium

to stimulate cholesterol biosynthesis. Such cell studies can be very useful in a patient with a suspected clinical diagnosis of RSH/SLOS but normal or equivocal blood sterol levels. The observation that full biochemical expression in RSH/SLOS cells requires cholesterol-deficient culture medium [Kelley, 1995; Honda et al., 1997] is especially important with regard to prenatal diagnosis using cultured cells. In general, cultures of villus cells and amniocytes cannot be established without cholesterol in the culture medium and, once established, often die when they are refed with cholesterol-depleted culture medium to bring out the diagnostic RSH/SLOS sterol profile. When grown in cholesterol-containing media, fibroblasts or lymphoblasts from RSH/SLOS homozygotes may be biochemically almost indistinguishable from control or, especially, RSH/SLOS heterozygote cells. Hence, direct sterol analysis of amniotic fluid or chorionic villus tissue remains the best method for prenatal diagnosis.

TREATMENT OF CHOLESTEROL DEFICIENCY OF RSH/SLOS SYNDROME

The impression of most geneticists is that the quality of life for RSH/SLOS patients is improved when a cholesterol-enriched diet is given. To remedy cholesterol levels as low as 1 mg/dl at diagnosis, many patients with RSH/SLOS have been treated with supplementary cholesterol following several different protocols [Abuelo, 1997; Elias et al., 1997; Irons et al., 1995; Nwokoro and Mulvihill, 1997]. In general, patients have been given from 50–100 mg/kg/day of cholesterol with or without a bile-acid supplement. In this issue, Elias et al. [1997] and Nwokoro and Mulvihill [1997] describe their experience with cholesterol and bile-acid treatment of a total of 12 patients with RSH/SLOS. Although experience with cholesterol supplementation is still quite limited and quantitative measures of improvement are few, the results have been encouraging. The most convincing evidence of benefit is the often striking improvement in weight and linear growth when cholesterol is added to the diet [Elias et al., 1997; Nwokoro and Mulvihill, 1997]. Anecdotally, improvement in behavior has also been impressive. However, objective measures of improved behavior are so far lacking. Other areas of apparent improvement, as described by Elias et al. [1997], include strength, endocrine function (rapid onset of delayed puberty), and immune function. Two "adverse" effects following cholesterol treatment have been excessive weight gain in several children [Nwokoro and Mulvihill, 1997] and acceleration of male pattern baldness in a 30-year-old RSH/SLOS man, coincident with a substantial rise in his serum testosterone levels.

One controversy about dietary treatment of RSH/SLOS concerns the use of bile-acid supplements. The rationale behind the use of a bile-acid supplement is twofold. First, normal bile-acid synthesis is deficient in RSH/SLOS and, therefore, cholesterol absorption from the gut may be impaired [Natowicz and Evans, 1994]. Second, suppression of endogenous bile-acid synthesis effected by some bile-acid supplements will spare cholesterol from conversion into bile acids. However, an important concern raised by Dr. Gerald Salen at the 1995 NICHD conference is that bile-acid supplements can downregulate tissue levels of LDL receptors, a principal determinant of cholesterol delivery to tissues of patients with RSH/SLOS. Thus, a rising cholesterol level in a patient given bile salts could be caused not only by increased intestinal absorption of cholesterol but also by decreased levels of LDL receptors. The paper by Ness et al. [1997] clearly shows that LDL receptor levels were greatly increased in the liver and brain of a severely affected RSH/SLOS patient, perhaps reflecting an attempt by cells to scavenge as much cholesterol as possible. Also of note is that in some patients treated with cholesterol alone, the blood cholesterol levels have remained essentially constant despite marked acceleration of growth. Only when the growth rate later slows does the blood cholesterol level begin to rise.

The uncertain interpretation of rising or nonrising cholesterol levels in RSH/SLOS patients underscores how much we have to learn about both normal and abnormal cholesterol metabolism before we can devise better therapies for RSH/SLOS patients. Important, also, will be very close monitoring of as many clinical and biochemical parameters as possible in the patients we treat. Toward this end, clinical consortia have been organized in the U.S., Italy, Northern Europe, Australia and Japan for developing formal studies of RSH/SLOS treatment. This organized approach to the study of RSH/SLOS will doubtless bring many new benefits in the near future.

TERATOLOGY OF RSH/SLOS AND DEFECTIVE CHOLESTEROL BIOSYNTHESIS

Another area in which knowledge about RSH/SLOS is seriously lacking is the relationship between cholesterol metabolism and abnormal morphogenesis. From a embryological standpoint, cholesterol has a dual role, serving not only as the precursor of all steroid hormones but also as an essential component of the plasma membrane and specialized subcellular membranes, such as the mitochondrial outer membrane. Although survival to term of RSH/SLOS fetuses having more than 80% of their sterol in the form of 7DHC may at first seem impossible, in a number of eukaryotes, especially in the plant kingdom, 7DHC rather than cholesterol is the predominant membrane sterol. Moreover, RSH/SLOS patients with the highest 7DHC levels at birth often have fewer malformations than some RSH/SLOS newborns with much lower 7DHC levels [Cunniff et al., 1996]. Nevertheless, because of the critical role of cell membranes in cellular nutrition and cell-cell interactions, the widespread morphological abnormalities in some RSH/SLOS patients are not suprising, if poorly understood at this time.

As noted earlier, many studies dating back more than 30 years describe RSH/SLOS-like malformations in rats treated with inhibitors of cholesterol biosynthesis [Barbu et al., 1984; Roux and Aubry, 1966; Roux et al., 1980]. In this issue, one of the few groups to explore the connection between deficient 7DHC reductase activity and abnormal morphogenesis summarizes their experiments exposing early rat embryos to BM 15.766, a rel-

atively specific inhibitor of 7DHC-reductase [DeHart et al., 1997]. One of their important findings was that cells in the cranial neural folds of exposed fetuses were abnormally shaped and had lost many cell-cell contacts. Identically exposed rat embryos sacrificed later had a high frequency of holoprosencephaly. Similar alterations in cell morphology and loss of cellular attachment are also evident in cultured RSH/SLOS fibroblasts and amniocytes within 24 hr of changing from normal culture medium to cholesterol-depleted medium (Kelley, unpublished observations). In contrast, cultured RSH/SLOS lymphoblasts grow quite well in suspension with or without cholesterol in the culture medium and reach 7DHC/cholesterol ratios as high as 2. Thus, whereas most cellular functions seem preserved when 7DHC replaces cholesterol, cell-cell interactions critical to normal morphogenesis appear to be very sensitive to cholesterol deficiency. In this regard, it is interesting to note how many of the RSH/SLOS internal malformations, such as endocardial cushion defects, abnormal pulmonary lobation, and Hirschsprung disease, can be explained, at least in part, by defective migration of cells.

The important role of cholesterol as the precursor of all steroid hormones probably contributes in some way to the genital malformations characteristic of RSH/SLOS. However, cell-cell interactions and other morphogenic processes unrelated to abnormal steroid metabolism may also be impaired. For example, because pituitary agenesis is relatively common in the rat model for SLOS [Roux et al., 1979], deficient hypothalamic-pituitary function may also play a role in the genital maldevelopment in some patients [Pankau et al., 1992]. Moreover, deficient steroidogenesis cannot explain the persistence of Müllerian structures in the more severely affected RSH/SLOS males. Thus, there may also be effects of 7DHC and low cholesterol levels on cell-cell interactions or other steroid-independent morphogenic processes in the urogenital anlage. Interestingly, in one adult with classical SLOS, a testicular biopsy showed a marked deficiency of Leydig cells [Hoefnagel et al., 1969], which could also explain the persistence of Müllerian duct derivatives in some RSH/SLOS patients.

RSH/SMITH-LEMLI-OPITZ SYNDROME: A NEW PARADIGM OF METABOLIC DISEASE

Since the conceptualization of inborn errors of metabolism early in this century by Sir Archibald Garrod [1908], rapid advances in the delineation of new metabolic diseases have often followed the wedding of laboratory technology with a change in our thinking about metabolic diseases. The discovery of phenylketonuria as a cause of mental retardation and the development of practical amino-acid chromatography immediately preceded the discovery of many new inborn errors of amino-acid metabolism. Similarly, the initial slow elucidation of organic acidurias by laborious chemical methods was soon followed by an explosion of discovery of new organic acidurias once practical gas chromatography became available to clinical laboratories. In the case of RSH/SLOS, the diagnostic technology, i.e., sterol and bile-acid gas chromatography-mass spectrometry, was here, but where to look for inborn errors of sterol biosynthesis was anyone's guess.

The discovery of cholesterol deficiency in RSH/SLOS has directed attention to a little thought about the paradigm of metabolic disease. That is, the biochemistry of SLOS is the best example yet of fetal abnormalities caused by defective biosynthesis of a metabolite that, unlike glucose, amino acids, and organic acids, cannot be transported across the maternal-fetal placental barrier. The inverse correlation of RSH/SLOS severity with blood cholesterol levels at diagnosis strongly suggests that the abnormal morphogenesis of RSH/SLOS is directly related to the deficiency of endogenous cholesterol synthesis, which cannot be remedied by the mother. Whereas the clinical metabolic maps for many groups of inborn errors of *catabolism*, i.e., organic acidurias, amino acidemias, storage diseases, etc., are essentially complete, only one other disease, mevalonic aciduria, of the long cholesterol *biosynthetic* pathway, is known. Similarly, only two defects are known among the many possible defects of biosynthesis of phospholipids, another class of compounds not supplied in sufficient amounts from the maternal circulation. An inability to be transported from mother to fetus probably applies to a great many fat-soluble metabolites. However, for almost all such pathways of obligate fetal lipid biosynthesis, no clinical deficiency syndromes are known. Although tools for the identification of inborn errors of lipid biosynthesis may not be available to most clinical laboratories, more important to realize is that we have not actively searched for inborn errors of lipid biosynthesis, in part for lack of knowing where to search. As we near the beginning of the second century of Garrod's "inborn errors of metabolism," we can hope that the many new biochemical and genetic lessons that RSH/SLOS has taught us will help us fill in these obvious lacunae in our knowledge of biochemical genetics.

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